

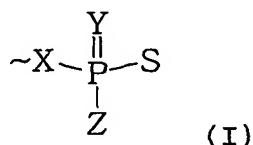
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CLAIMS

1. A hairpin polynucleotide, having a loop and a stem region, characterised in that a sulfur-based nucleophile is attached to an internal nucleotide in the hairpin through a linker to enable binding to a solid support.

2. The hairpin polynucleotide as claimed in claim 1 wherein the internal nucleotide is present in the loop of the hairpin.

3. The hairpin polynucleotide as claimed in claim 1 or claim 2 wherein the sulfur-based nucleophile is a thiol (~SH) or a moiety of the formula (I):



(wherein ~ denotes the bond or linker connecting the sulfur-based nucleophile to the remainder of the polynucleotide; X represents an oxygen atom, a sulfur atom or a group NR, in which R is hydrogen or an optionally substituted C₁₋₁₀ alkyl; Y represents an oxygen or a sulfur atom; and Z represents an oxygen atom, a sulfur atom or an optionally substituted C₁₋₁₀ alkyl group).

4. The hairpin polynucleotide as claimed in claim 3 wherein X is oxygen, sulfur, or NH.

5. The hairpin polynucleotide as claimed in either claim 3 or claim 4 wherein Y is oxygen.

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6. The hairpin polynucleotide as claimed in any one of claims 3 to 5 wherein Z is an oxygen or sulfur atom or a methyl group.

5 7. The hairpin polynucleotide as claimed in any one of claims 3 to 6 wherein the sulfur-based nucleophile is a thiophosphate moiety.

8. The hairpin polynucleotide as claimed in any one
10 preceding claim comprising a DNA or an RNA.

9. The hairpin polynucleotide as claimed in claim 8 wherein the thiophosphate moiety is attached to a modified nucleotide.

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10. The hairpin polynucleotide as claimed in claim 9 wherein said modified nucleotide is an abasic nucleotide.

11. A hairpin polynucleotide as claimed in any one
20 preceding claim wherein said linker is selected from the group comprising polyethylene glycol of formula $-(CH_2-CH_2-O)_m$ (wherein m is an integer of from about 1 to about 600), dextrose, peptides, nucleic acids or modified or unmodified chain of formula $-(CH_2)_n$ (wherein n is an integer of from
25 about 1 to about 1,500).

12. A hairpin polynucleotide as claimed in claim 11 wherein the linker comprises a modified chain of formula $-(CH_2)_n$ wherein n is less than 100 and the modifications comprise
30 the replacement of one or more than one CH_2 units for functional groups selected from the group comprising ketones, esters, amines, amides, ethers, thioethers, sulfoxides, sulfones, alkene, alkyne, aromatic or heteroaromatic moieties or cyclic aliphatic moieties.

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13. The hairpin polynucleotide as claimed in claim 12 wherein the modified chain comprises one or more amide bonds and one or more carbon-carbon triple bonds.

5 14. The hairpin polynucleotide as claimed in any one of claims 11 to 13 wherein the linkers comprises a propargylamino unit.

10 15. The hairpin polynucleotide as claimed in any one preceding claim comprising a first target polynucleotide attached to the 5' end of the hairpin.

16. The hairpin polynucleotide as claimed in claim 15 wherein said first target polynucleotide is genomic DNA.

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17. The hairpin polynucleotide as claimed in claim 15 or claim 16 wherein said first target polynucleotide is human genomic DNA.

20 18. The hairpin polynucleotide as claimed in any one preceding claim comprising a primer attached to the 3' end of the hairpin.

25 19. The hairpin polynucleotide as claimed in any one preceding claim wherein the stem comprises a 5 to 25 base pair double-stranded region.

30 20. The hairpin polynucleotide as claimed in any one preceding claim wherein the loop comprises 2 or more non-hybridised nucleotides.

21. The hairpin polynucleotide as claimed in any one of claims 1 to 19 formed from 2 or more separate polynucleotides with complementary regions and a loop which
35 comprises a non-nucleotidic connecting moiety.

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22. The hairpin polynucleotide as claimed in claim 21 wherein said linker moiety comprises PEG.

5 23. A method of making a hairpin polynucleotide, as defined in any one preceding claim, comprising attaching the sulfur-based nucleophile to said internal nucleotide before, after or during formation of the hairpin polynucleotide.

10 24. An array of hairpin polynucleotides as defined in any one of claims 1 to 22 immobilised on a surface of a solid support.

15 25. The array as claimed in claim 24 which is a single molecular array.

26. The array as claimed in either claim 24 or claim 25 wherein said solid support comprises glass, ceramics, glass silicon or plastics.

20 27. The array as claimed in any one of claims 24 to 26 wherein said solid is a glass slide.

25 28. The array as claimed in any one of claims 24 to 27 wherein the hairpin polynucleotides are immobilised by covalent bonding.

29. The array as claimed in claim 28 wherein said covalent bonding is formed between the sulfur-based nucleophile and an electrophilic group displayed on the surface of the solid support.

30 30. The array as claimed in claim 29 wherein said electrophilic group is attached to a silicon atom.

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31. The array as claimed in claim 30 wherein said surface is modified so that it in part comprises a silane of formula $R_nSiX_{(4-n)}$ (where R is an inert moiety that is displayed on the surface of the solid support, n is an integer of from 1 to 4 and X is or comprises a reactive leaving group).

32. The array as claimed in any one of claims 29 to 31 wherein the electrophilic group is formed from bromoacetamide functionality.

33. A method of making an array as defined in any one of claims 24 to 32 comprising the steps of:

- (i) preparing a plurality of hairpin polynucleotides as defined in any one of claims 1 to 22; and
- (ii) immobilising said hairpin polynucleotides to a surface of a solid support so as to form said array.

34. The method of making an array of hairpin polynucleotides as claimed in claim 33 comprising an additional subsequent step of ligating a second target polynucleotide to each hairpin polynucleotide after they have been immobilised to the surface of the solid support.

35. The method of making an array as claimed in claim 34 wherein said second target polynucleotide is genomic DNA.

36. The method of making an array as claimed in claim 34 or claim 35 wherein said second target polynucleotide is human genomic DNA.

37. The method of making an array as claimed in any one of claims 33 to 36 wherein either or both of said first and second target nucleotides is or are attached to the hairpin polynucleotides by ligating one strand of the target

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nucleotide in the form of a double-stranded DNA to the hairpin polynucleotide and removing the other strand after the ligation.

5 38. A device comprising an array as defined in any one of claims 24 to 32.

39. Use of the device of claim 38 in the interrogation of said hairpin polynucleotides.

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40. The use as claimed in claim 39 in an analytical procedure to determine the sequence of the first target polynucleotide.

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